THE EARLY GROWTH RESPONSE OF ETIOLATED OAT AND CORN SEEDLINGS TO DECAPITATION AND DESEEDING

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(Received May, 1988; revised and accepted July, 1988)

ABSTRACT

Boul, H. L. & Leung, D. W. M. (1988). The early growth response of etiolated oat and corn seedlings to decapitation and deseeding. *New Zealand Natural Sciences* 15: 11 - 16.

The growth of etiolated oat (*Avena sativa* L. cv. 'Terra') and corn (*Zea mays* L. cv. 'Stowell's Evergreen') seedlings were re-evaluated to determine whether growth responses are similar in the two species. The growth response of the mesocotyl and coleoptile was recorded following coleoptile tip decapitation, removal of the whole coleoptile and primary leaves, or deseeding. The use of infrared photography avoided the artifacts associated with safelights of visible wavelength. The response of the coleoptiles of both species to the treatments was similar, but mesocotyl growth response to all treatments was slower in oat than in corn.

KEYWORDS: *Zea* - *Avena* - coleoptile - mesocotyl - growth - infrared photography.

INTRODUCTION

Since the work of Darwin & Darwin (1880) etiolated cereal seedlings have been a model system for the study of growth control, but the mechanism of control remains a topic for debate (for review see Jackson & McWha 1984).

Workers investigating growth of etiolated corn and oat seedlings have generally assumed that both species possess the same mechanism of control. Jackson & McWha (1984) outlined a number of morphological and biochemical differences between the two species which suggest that the control mechanism in corn and oat may be different.

The principal methods used to examine growth of seedlings have been destructive harvesting (Iino & Carr 1982, Momonoki *et al.* 1983) and visible light photography (Skoog 1937). Destructive harvesting is unsuitable because the growth profile of a single sample can not be followed, while visible light photography is unacceptable as the seedling response threshold to even green light, is at a level below that at which it is possible to work (Iino & Carr 1981).

The aim of this study was to examine the growth profile of oat and corn seedlings treated under identical conditions. Growth was assessed using infrared (i.r.) photography, which enabled the short term time-course of growth responses to be studied in detail without compromising the need for total darkness.

MATERIALS AND METHODS

PLANT MATERIAL

Corn seeds (*Zea mays* L. cv. 'Stowell's Evergreen', Ferry Morse Seed Co., Mountain View, California, USA.) were soaked in aerated deionised water for 24 h, then sown in moist vermiculite in plastic boxes loosely covered with aluminium foil to maintain humidity. When seedlings attained a total length of ca. 70 mm they were transferred to the jigs described below. Oat seeds (*Avena sativa* L. cv. 'Terra', Ocean View Seeds Ltd., New Plymouth N.Z.) were individually placed between folded blotting paper strips in glass tubes (50 x 4 mm i.d.) in a similar manner to that of Mer (1951). These holders were then placed in racks in plastic boxes, with

approximately 15 mm of deionised water in the bottom, and loosely covered with aluminium foil. The seedlings were allowed to grow until they were ca. 20 mm in total length when they were removed from the holders and transferred to the jigs described below. With both species seedling size was chosen so that experiments were performed during the 'grand' phase of mesocotyl growth. At all times the plants were grown in darkness at 24°C.

GROWTH JIGS

Jigs (Fig. 1) were constructed of perspex, and consisted of a black backplate with 280 x 6 x 6 mm strips cemented on to form 8 channels (280 x 12 x 6 mm). A recess was drilled into the backplate 50 mm from the lower edge of each channel to accommodate the seed. A series of six 1 mm holes at 28 mm centres was drilled in the backplate above the seed recess to facilitate air circulation. Two glass plates 150 x 50 mm and 230 x 150 mm were used as covers; the smaller plate covering the roots and seeds, and the larger covering the shoots. Plants were placed in the jigs by placing moist absorbent cotton wool in the channels below the recess and then gently laying the seedling over this so that the seed lay in the recess and the roots were in contact with the cotton wool. The coleoptilar nodes (and the mesocotyl bases in deseeding experiments) were then marked using waterproof drawing ink (Faber). The glass covers were then fitted and secured with elastic bands. Spacers of 14 mm thickness were then slipped under the bands and the whole assembly placed vertically in the growth box. The first photographic exposure was made within 1 h of placing seedlings in the jigs.

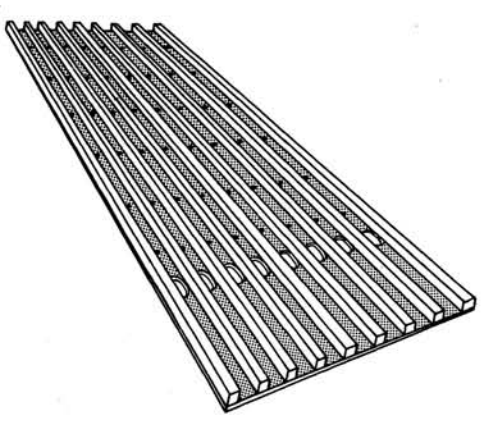

Figure 1. General sketch of growth jig.

GROWTH BOX

The growth box shown in Fig. 2 was constructed of black perspex. It was designed so that seedlings were supplied with a continuously changing aerated-water supply and an atmosphere of constant composition: flowing tap water (200 - 250 ml min^{-1}) was aerated behind a 30 mm baffle and allowed to flow across the base of the box to the drain, thus maintaining a constant depth of 16 mm. Compressed air from the laboratory supply was filtered through activated charcoal and potassium permanganate, and then bubbled through tapwater to increase its humidity. The air was introduced into the box through a series of small holes in the box sides. The same air supply was used to aerate the water supply. The lid of the box was a loose fitting flat plate that allowed free egress of air.

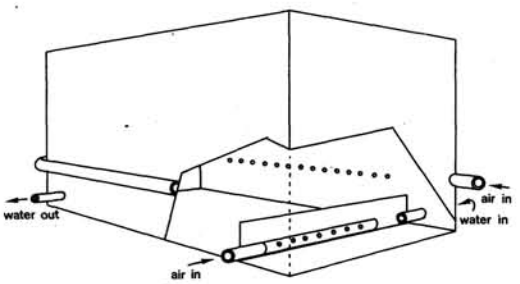

Figure 2. General sketch of growth box. The main dimensions are 165 x 250 x 290 mm.

SAFELIGHTS

Safelights were constructed of i.r. light emitting diodes (LEDs) (Phillips CQY89A or equivalent). One bank was portable; consisting of 16 LEDs running at a current of 13 mA from a 9 V battery. Two banks of 20 LEDs running at 24 mA, and two banks of 24 LEDs running at 47 mA were powered by a 12 V supply and set up for use as photographic light sources. These

were also used to illuminate the work area. Manipulations were performed at least 10 cm from the large banks and at least 5 cm from the portable bank. The i.r. radiation was visualized using a head mounted 'Find-R-Scope' (FJW Industries, Mount Prospect, Illinois, USA.), as used by Iino & Carr (1981).

TREATMENT OF SEEDLINGS

After four exposures, jigs were removed from the growth box for treatment. In each jig alternate seedlings were treated, with the remainder serving as controls. Seedlings were treated by removing the glass covers and excising the appropriate tissue with a scalpel. Tip decapitations were performed using a jig to ensure even 4 mm tips were excised from each seedling. Whole coleoptiles and primary leaves were excised such that the coleoptilar nodes remained intact. The coleoptilar nodes and mesocotyl bases were re-marked as necessary and the jigs reassembled and placed back in the growth box.

MEASUREMENT

Measurement was accomplished photographically. Exposures (7 s, f 4) were made at appropriate time intervals onto i.r. sensitive film (Kodak High Speed Infrared 2481), using the four large LED banks as the radiation sources. Measurements were made from images enlarged 2 - 8 times.

RESULTS AND DISCUSSION

In both species the initial effect of decapitation of the coleoptile tip was to reduce coleoptile growth. In oat (Fig. 3a) this occurred after about 1 h, while in corn (Fig. 4a) the response was more rapid. A period of approximately 3 h of little growth was followed by resumed growth at a rate almost identical to that of the controls. The initial reduction of growth and subsequent recovery is a well known phenomenon known as 'regeneration of the physiological tip' (Went & Thimann 1937 p. 25). This 'regeneration' has been reported as less than complete in oat, resulting in a growth rate that is lower than that before treatment (Dolk, quoted by Went & Thimann 1937 p. 25, and Thimann & Bonner 1933). Mer (1972) produced similar results to

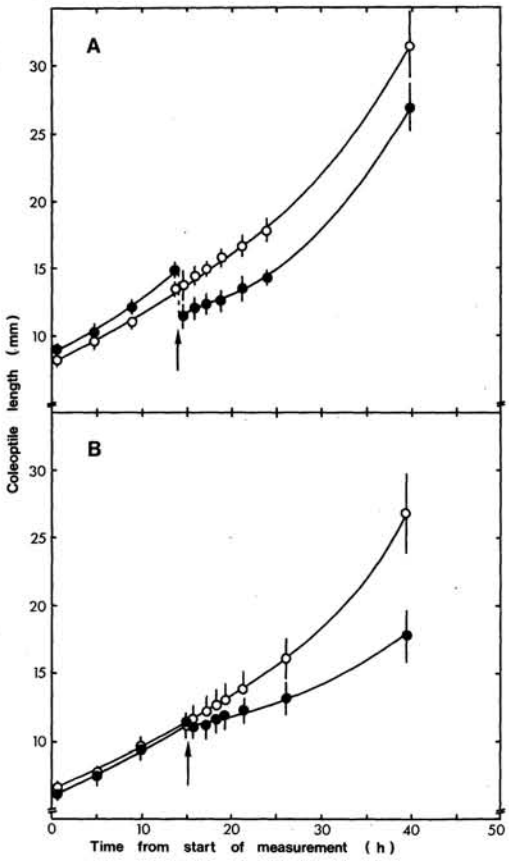

Figure 3. Response of oat coleoptiles to a) coleoptile tip decapitation (n=11), and b) deseeding (n=11). (●) Treated plants, (○) control plants. Vertical bars represent + and/or - the standard error of the mean. The arrow indicates the time of treatment.

those cited here, and attributed the anomaly to 'safelight' effects. Mer, in his work however, decapitated very small tips (0.4 mm) without showing whether such tips contained the 'physiological tip', and he measured growth 3 days after decapitation.

Mesocotyl response to decapitation differed between the species. In corn (Fig. 6a) the response was rapid (< 1h) and was manifested as a 50 % reduction in growth rate. This result was not dissimilar to that of Inge & Loomis (1937) who found that three decapitations at 8 h intervals resulted in a reduction in corn mesocotyl length to 40% of that of the controls. It should be noted that these workers used red safelights.

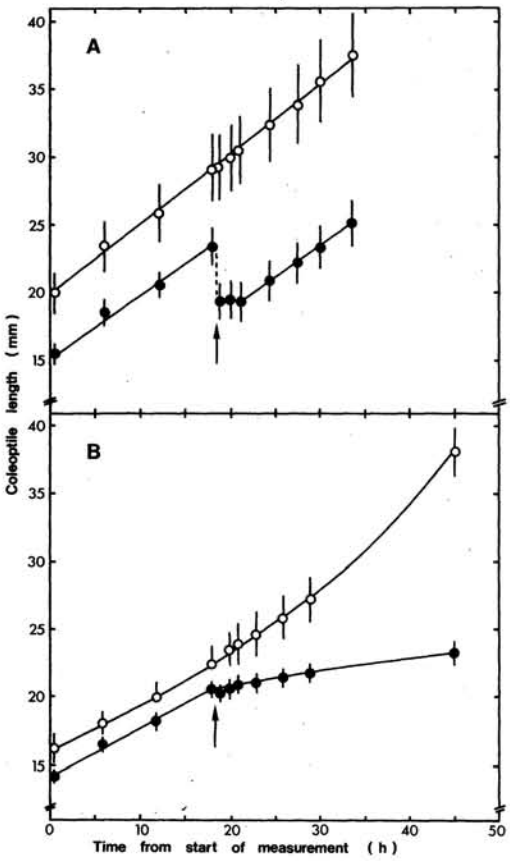

Figure 4. Response of corn coleoptiles to a) coleoptile tip decapitation (n = 11), and b) deseeding (n = 12). Symbols as for Fig. 3.

Iino & Carr (1982) found a similar reduction in mesocotyl length to that reported here, in seedlings measured 8 h after decapitation. In oat (Fig. 4a) the growth rate slowly declined over 24 h, although little change was apparent during the 5 h following decapitation. In neither species did the mesocotyl show signs of a recovery in growth as was seen in the coleoptile. This result does not agree with that of Mer (1951), who, by careful work in absolute darkness showed that oat mesocotyls were unaffected by coleoptile tip decapitation. Unfortunately Mer treated his seedlings at an age at which the mesocotyl growth rate was declining, and in addition did not measure the plants until 48 h after mesocotyl growth had stopped. His results, therefore, cannot be said to contradict those reported here.

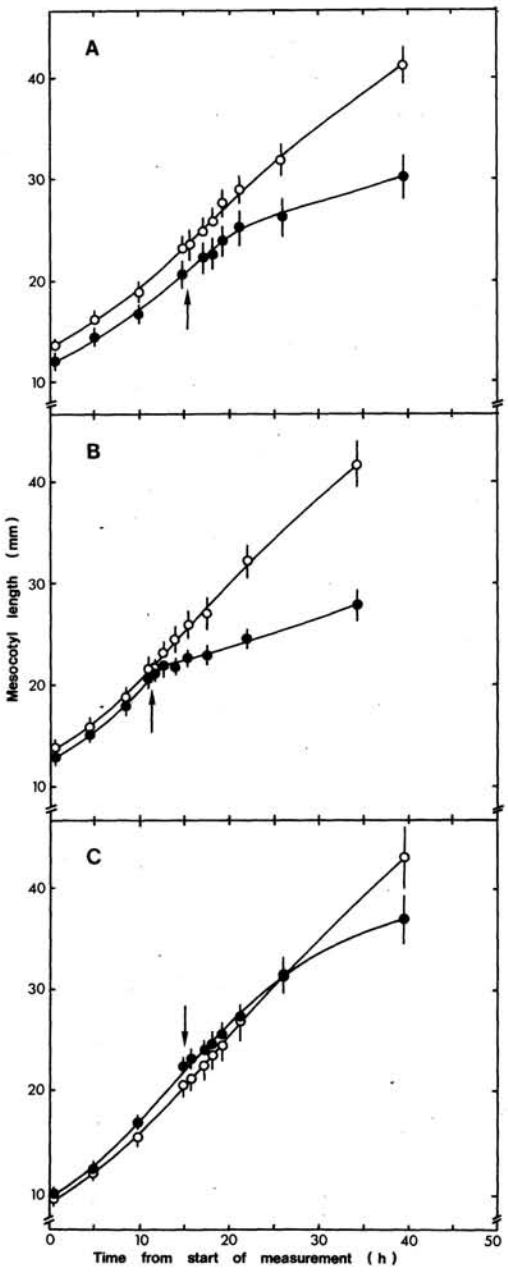

Figure 5. Response of oat mesocotyls to a) coleoptile tip decapitation (n = 11), b) removal of whole coleoptile and primary leaves (n = 12), and c) deseeding (n = 11). Symbols as for Fig. 3.

There is nothing in the coleoptile data for either species to suggest that 'regeneration of the

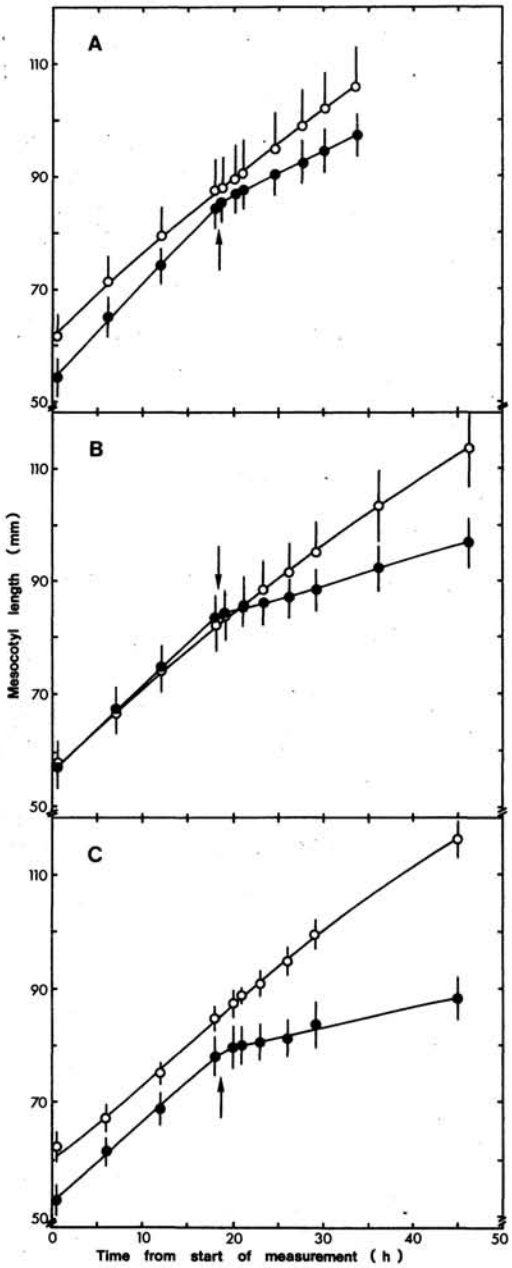

Figure 6. Response of corn mesocotyls to a) coleoptile tip decapitation (n = 11), b) removal of whole coleoptile and primary leaves (n = 12), and c) deseeding (n = 12). Symbols as for Fig. 3.

physiological tip' is anything but complete. Van Overbeek (1935) has suggested that the suppression of mesocotyl growth following tip removal results from an inability of the regenerated tip to supply adequate auxin to this tissue. If van Overbeeks' postulate is to be accepted however, a different pattern of mesocotyl response to detipping would be expected i.e. a cessation of growth after a lag phase, followed by a resumption of growth at a lower rate (proportional to the available auxin) after regeneration of the tip.

Removal of the whole coleoptile and leaves produced a similar reduction in growth rate of the mesocotyls of oat and corn. The response is rapid (< 1h) in both species, and resulted in a steady reduced growth rate for at least 20 h following treatment. In oat (Fig. 5b) this rate is ca. 33% that of the controls, while in corn (Fig. 6b) it is ca. 25%. Mer (1972) found that in oat a similar treatment significantly promoted mesocotyl elongation after six days, although examination of his data suggests that an initial inhibition occurred.

Deseeding initially stopped growth in the coleoptiles of both species. In corn (Fig. 4b) growth resumed at a low rate after a lag of approximately 2 h. The control coleoptiles entered the 'grand phase' of growth during the experimental period but the treated coleoptiles did not. The result of deseeding on oat coleoptiles (Fig. 3b) was similar to that of corn, however, the treated oat coleoptiles did enter the 'grand phase' at the same time as the controls, although the magnitude of the response was much reduced. This is quite different to the result reported by Skoog (1937), who found that coleoptiles from deseeded oat plants showed an elongation of about 80% of the controls after 6 h and had completely ceased growth after 16 h. The shape of his growth curve and the method of measurement suggest that this result is due more to an effect of light than to deseeding.

There was a marked difference between the species in the response of the mesocotyl to deseeding. In corn (Fig. 6c) the response was identical to that elicited by removing the whole coleoptile (i.e. rapid drop in growth rate to ca. 25% of the control rate). In oat (Fig. 5c) the mesocotyl reacted slowly. After 4 h the growth rate was the same as in the control plants. Ten hours after treatment the growth rate of the

treated mesocotyls was ca. 66% that of the controls; this rate continued to decline over the remainder of the experimental period, although significant growth was still occurring 24 h after treatment. The 33% reduction in total shoot length 24 h after deseeding found in this investigation is similar to the 45% reduction reported by Momonoki *et al.* (1983) in the same variety of corn.

The major difference between oat and corn was found to be in the response time of the mesocotyls to the various treatments. Corn mesocotyls responded rapidly to all treatments (< 1h), whereas oat mesocotyls showed considerable lag phases. It should be noted that in none of the experiments did the growth rate of the mesocotyl of either oat or corn show any tendency to recover after treatment, whereas the coleoptiles recovered in all cases except the deseeded corn.

The coleoptile of both species responded to the treatments considered in a similar manner, while the response of the mesocotyl was different. If the 'auxin' ('auxin' refers to free IAA and conjugates, and precursors of auxin etc.) theories of growth (as reviewed by Jackson & McWha 1984) are accepted, then the data can be explained by an auxin transport hypothesis.

If the route of auxin transport is assumed to be from seed, to coleoptile tip, to coleoptile, to mesocotyl, it can be seen that the further the mesocotyl is from the excised part on the transport route, the longer the response time will be. In addition, the seed to tip transport must be rapid, as the oat coleoptile responds to deseeding rapidly. In oats therefore, the data suggests a large pool of auxin in the coleoptile unit which must be exhausted before any effect is manifested in the mesocotyl.

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